



Transposon Mutagenesis Update and Marker Exchange Deletion Mutants of Na^+/H^+ Antiporters

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Purpose - Transposon Mutagenesis

- To expand the random transposon mutant library in *Desulfovibrio vulgaris* Hildenborough.
 - To use wild type *D. vulgaris* Hildenborough containing the megaplasmid for the mutagenesis.
 - To expedite the identification of the transposon insertion site.

New Protocols

- Since the JW375 strain used as the parental strain for the first transposon mutants lacked the megaplasmid, the wild type *D. vulgaris* Hildenborough is now being used as the parental strain.
 - Gentamicin replaced nalidixic acid to select against the *E. coli* donor in the conjugation.
 - Two new transposon integration site identification methods were developed:
 1. Random primer nested PCR.
 2. Direct genomic sequencing.

Mutations

Table 1. Genes disrupted by the transposon, sequence adjacent to the interruption, and operon information.

Progress

- Generation of transposon mutants in wild-type *D. vulgaris* Hildenborough has been accomplished.
 - Two high throughput methods of insertion site determination have been developed.
 - A manuscript on the transposon libraries in *D. vulgaris* Hildenborough and *Desulfovibrio desulfuricans* G20 is being written

Purpose – Marker Exchange Deletion Mutagenesis

- To create mutants in *Desulfovibrio vulgaris* Hildenborough which lack a specific gene.
 - To create mutants in the Na^+/H^+ antiporter genes.
 - To determine the role each Na^+/H^+ antiporter has in Na^+ homeostasis.

Deletion Procedure

1. Design primers for the three PCR amplicons.
 2. Perform the first three PCR reactions.
 3. Gel purify products.
 4. Combine the three PCR amplicons into the mutagenic cassette using another PCR reaction.
 5. Gel purify product.
 6. Clone the mutagenic cassette into a vector for transfer to *D. vulgaris* Hildenborough.
 7. Confirm the arrangement and sequence of the cassette by sequencing the plasmid.
 8. Transform *D. vulgaris* Hildenborough with the mutagenic vector using electrotransformation.
 9. Screen the transformants with PCR.
 10. Perform single-colony isolation of a positive transformant.
 11. Perform Southern analysis on a single colony to confirm mutation.

Mutations

Table 2. The status of genes to be deleted by Marker Exchange Deletion Mutagenesis

| JW# | Gene | VIMSS# | pMO# | ORF # | DVU # | Colonies | PCR confirmed | Southern Confirmed | Northern Confirmed | uptag (forward) sequence | downtag (reverse) sequence |
|-----|---------|--------|------|-------|-------|----------|---------------|--------------------|--------------------|--------------------------|----------------------------|
| 385 | cycA | 208692 | 385 | 4250 | 3171 | Y | Y | Y | N | TCTTGTAGATGTCCTCCG | TAGTTTCACTGACTCTAG |
| 380 | mnhA | 209370 | 380 | 5368 | 0434 | Y | Y | Y | N | CAGGACTGAGATGCGATAC | ATCTCAGTAACTAGGGACC |
| 381 | nhaD | 208955 | 381 | 4673 | 0027 | Y | Y | Y | N | ATCTACTGACAGACGGAGC | ACGCTCTAAATAGTACCCC |
| 382 | nhaC-1 | 209317 | 382 | 5281 | 0381 | Y | Y | Y | N | CGGAGACACCCGACATAG | TTCTGCGAGCCGTAGTGA |
| 383 | nhaC-2 | 208626 | 383 | 4140 | 3108 | Y | Y | Y | N | GCCGACAGACCGTGA | AGCTCTGGACACTATACAC |
| - | cytK | 206086 | - | 0034 | 0663 | N | N | N | N | AGTCCTGCAAGCTGTACA | ATAGTGAACTACCTGAGC |
| 386 | ung | 209329 | 386 | 5299 | 0393 | Y | Y | Y | N | TGCTCTGCACGGTAGAGCT | CTATTCCTAACGTCTAGCG |
| 387 | pnp | 209440 | 387 | 5499 | 0503 | N | N | N | N | CGACGCCAAAGTACGTAGA | CGTAGCTGAGAATGAAACCC |
| 388 | ung G20 | 392942 | 388 | - | - | Y | N | N | N | | |
| 389 | nhaD2 | 207844 | - | 2356 | 2889 | N | N | N | N | TAGCGAGATTTGACGTAGC | CTGTAACGATCATAGAGCG |
| 390 | rpoH | 207035 | 390 | 1584 | 1558 | N | N | N | N | ACGATCCTAAAGTACCCAGG | GACCGAGACCTGATCAGAG |

Future Work

- To study the effects the Na^+/H^+ antiporter mutations have on the growth of *D. vulgaris* Hildenborough under different Na^+ concentrations and pH levels.
 - To perform Northern analysis on the Na^+/H^+ antiporter mutants for each of the Na^+/H^+ antiporter genes.
 - To study the effects the Na^+/H^+ antiporter mutations have on the growth of *D. vulgaris* Hildenborough under different nitrate concentrations.
 - To expand the transposon mutant library in wild-type *D. vulgaris* Hildenborough and *D. desulfuricans* G20.